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Production of Arrayed and Rearranged cDNA Libraries for Public Use

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August 30, 2005

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This work was performed under the auspices of the U.S. Department of Energy by University of California, Lawrence Livermore National Laboratory under Contract W-7405-Eng-48.

Production of Arrayed and Rearranged cDNA Libraries for Public Use.

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August 24, 2005

Prepared in partial fulfillment of the requirements of the Office of Science, Department of Energy's Community College Institute of Science and Technology Program under the direction of Christa Prange in the Biosciences Division at Lawrence Livermore National Laboratory.

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ABSTRACT

Production of Arrayed and Rearranged cDNA Libraries for Public Use. KRIS RASMUSSEN (Merced College, Merced, CA 95340) CHRISTA PRANGE (Lawrence Livermore National Laboratory, Livermore, CA 94550)

Researchers studying genes and their protein products need an easily available source for that gene. The I.M.A.G.E. Consortium at Lawrence Livermore National Laboratory is an important source of such genes in the form of arrayed cDNA libraries. The arrayed clones and associated data are available to the public, free of restriction. Libraries are transformed and titered into 384-well master plates, from which 2-8 copies are made. One copy plate is stored by LLNL while others are sent to sequencing groups, plate distributors, and to the group which contributed the library. Clones found to be unique and/or full-length are rearranged and also made publicly available. Bioinformatics tools supporting the use of I.M.A.G.E. clones are accessible via the World Wide Web.

INTRODUCTION

The annotation of the human genome was completed in April of 2003 [1], spelling out every nucleotide in our DNA. Researchers are now identifying the genes found within our genome and deciphering the functions of the proteins they encode. The I.M.A.G.E. (Integrated Molecular Analysis of Genomes and their Expression) Consortium at Lawrence Livermore National Laboratory (<http://image.llnl.gov>) is a provider of high-quality arrays of cDNA clones and their associated bioinformatics tools to the public for use in gene research [2]. cDNA libraries derived from tissues of human, mouse, rat, cow, *Fugu*, zebrafish, *Xenopus laevis*, and *X. tropicalis* are arrayed, frozen, and shipped to distributors and sequencers for sale and study.

METHODS

Library Transformation

All libraries received by I.M.A.G.E. are oligo-dT primed and directionally cloned by the supplier with a focus on full-length gene enrichment without the loss of rare/long transcripts. Libraries are currently constructed by the University of Tokyo, Express Genomics and Open Biosystems and arrive packed in dry ice. The cDNA is transformed into competent *E. coli* via electroporation and 10% glycerol stocks are created for long term storage at -80C. The libraries are plated onto LB agar containing the appropriate antibiotic and grown overnight (Fig. 1).



Plate Filling

Lidded plates containing 384 microtiter wells are filled with LB broth containing the appropriate antibiotic and 1ml/L of ferrichrome (Fig. 2). Ferrichrome is added to reduce the incidence of phage (a bacteria-infecting virus) contamination by binding to the FhuA receptor of *E. coli* and preventing the binding of T1, T5, and Phi-80 phages [3].

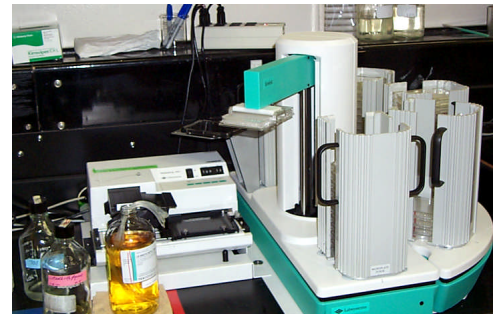


Figure 2. Plate filler.

Colony Picking

A Norgren Systems robotic picker is prepared for picking and placing colonies by first initializing the stages that hold the agar and microtiter plates and by sterilizing all the needles, which involves a water rinse followed by heat sterilization in a coil. The agar plate containing transformed colonies is placed on the left side of the picker and is scanned by a camera (Fig. 3). A computer program analyzes the image for colonies conforming to preset standards (i.e. diameter, proximity to other colonies). When prompted, the picker pierces the target colony with a needle and then transfers the colony into a well in a broth-filled 384-well plate on the right side of the picker. The 20 needles are arranged in a rotating ring so that picking, placing, and sterilization can occur simultaneously. Colonies are picked and placed until the requested number of plates is filled. These are labeled “replica 0” or R0 plates and are incubated over night at 37C. The labels have bar codes that contain library information and plate number. The picked plates are discarded.

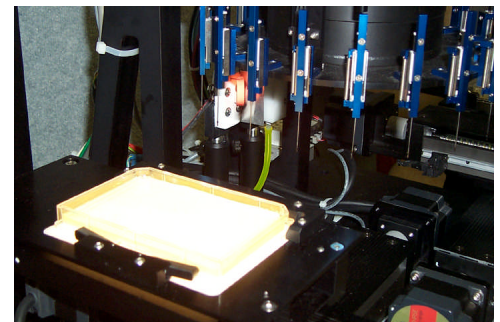


Plate Scoring

The next morning, the R0 plates are scored into the computer. Scoring involves scanning a plate's bar code and holding the plate up to the light to visually check each well for *E. coli* growth. The well will appear turbid if growth is occurring. If a well appears as a no-grow, the corresponding well on the computer is marked and stored for reference. The plate is discarded if 30 or more wells fail to grow.

Plate Replication

After scoring, the R0's are ready for replicating. Depending on demand for a particular library, up to eight replicas per R0 will be made. All replicas, including the R0's, are bar-coded for identification and tracking through the I.M.A.G.E. pipeline database. Phage-check plates are also made for QC purposes. The phage-check plates are *E. coli* lawns grown on LB agar. If a phage is present, it will lyse the cells and appear as a clear circle in the lawn. The R0's are loaded sequentially into trays, which can hold up to eight plates each. Each tray is loaded from top left to bottom left and then top right to bottom right. Once the R0 trays are loaded, the replicas and/or phage plates are loaded onto their trays. The Flexys computer is turned on and the robot is initialized while fresh 70% ethanol is added to the needle bath. The R0 trays are then loaded into the tray carrier, followed by the replica trays. When all the trays are loaded into the carrier, the first tray of R0's is robotically moved into place on the bed inside the Flexys, followed by the first replica tray. A 384-pin head drops into a sonicating ethanol bath, followed by heat sterilization. The pin head dips into the first R0 and then into the first replica. It then returns to the same R0, dips in, and moves to the next replica. This is repeated until all

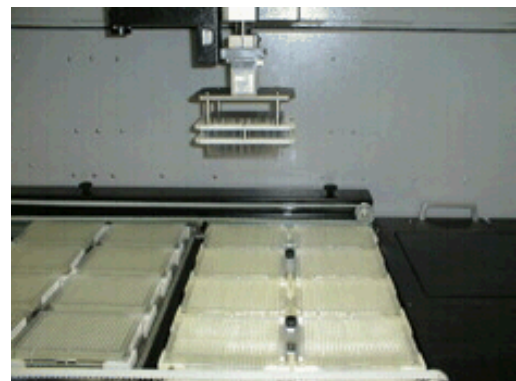


Figure 4. Plate replication.

replicas/phage plates have been inoculated. The pin head then returns to the ethanol bath and heat sterilization. The replica tray is returned to the tray carrier and the next replica tray is moved into place. The pin head is sterilized again and the process repeats until all R0's have been replicated. All the trays are unloaded after replication and the R0's are sealed with foil tape on a Brandel sealer and frozen at -80C. The other replicas, except for the R3's, are also sealed. All the replicas are incubated overnight and the R3's are scored against the R0's for corresponding no-grows. If there are 10 or more deviations, testing is done to determine the source of the errors. The R3's are then sealed and stored by I.M.A.G.E. at -80C. The other copies are shipped off to distributors, sequencers, and to the group donating the tissue for the library, if they requested copies.

Clone Rearranging

The sequencers will run samples from their plate copy to get the DNA sequences of those genes. All sequences are immediately submitted to Genbank, a public sequence repository at the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). Should analysis predict that the gene is potentially full-length, a request for a full 384-well plate of clones to be rearranged from the same species and vector is made by the sequencer. It is possible that the clones may be from different libraries. The request includes the plate numbers and addresses of the desired clones and when enough clones are requested, a rearrange plate is made using the Norgren Systems picker and custom rearranging software. The requested R3 source plates are pulled from the freezers and thawed for 2-3 hours. The script containing the clone request is accessed by the picker's computer and displays information regarding which plate number and well address is next to be picked from. The clone is picked and then placed into the rearrange plate. When the rearrange is complete, an output file is generated, listing each clone's original

location and its rearranged location. The R3's are reracked in the freezers and the rearrange plate (R0) is grown overnight. This plate is replicated according to the number of copy requests and grown overnight. The R0 and R3 plates are racked in the freezer and the other copies are shipped to the sequencing group and distributors.

CLONE USE BY RESEARCHERS

As mentioned earlier, clones may be purchased from distributors for unrestricted use. Many researchers will use the clones in protein expression studies to figure out what specific proteins are being coded for. They may also express large amounts of that protein for use in other experiments, for example to determine the structure of that protein or to figure out potential interaction partners like other proteins or DNA. I.M.A.G.E. clones are also used in microarray experiments to study the expression levels or regulation of many genes under different conditions. Clones arrayed by I.M.A.G.E. have been part of the Cancer Gene Anatomy Project [4], the Mammalian Gene Collection [5], the Zebrafish Gene Collection [6], the *Xenopus* Gene Collection [7] as well as other model organism study projects.

ACKNOWLEDGMENTS

This internship was conducted at Lawrence Livermore National Laboratory. I thank the U.S. Department of Energy, Office of Science for giving me the opportunity to participate in the CCI program and for the chance to have an incredible, real world lab experience. Special thanks go to my mentor, Christa Prange, for her knowledge and guidance. I also thank Kirsten

Schreiber and Nicole Shapiro for training me on the various robotics and for leading me through all the production steps.

REFERENCES

- [1] “The Human Genome Project Completion: Frequently Asked Questions”, [Online document] [Cited 2005 August], Available HTTP: <http://www.genome.gov/11006943>

- [2] “I.M.A.G.E. Consortium Goals”, [Online document] [Cited 2005 August], Available HTTP: <http://image.llnl.gov/image/html/igoals.shtml>

- [3] Muse, Michael, “Ferrichrome Incubation as a Means to Reduce Phage Titer”, [Online document] [Cited 2005 August], Available HTTP: <http://www.fruitfly.org/DGC/phage.html>

- [4] “Collaborating on Public Cancer Data”, [Online document] [Cited 2005 August], Available HTTP: <http://www.ncbi.nlm.nih.gov/projects/CGAP/>

- [5] “Mammalian Gene Collection”, [Online document] [Cited 2005 August], Available HTTP: <http://mgc.nci.nih.gov/>

- [6] “Zebrafish Gene Collection”, [Online document] [Cited 2005 August], Available HTTP: <http://zgc.nci.nih.gov/>

- [7] “Xenopus Gene Collection”, [Online document] [Cited 2005 August], Available HTTP:
<http://xgc.nci.nih.gov/>